COMPOSITION OF MILK FROM THE PALLID BAT (*ANTROZOUS PALLIDUS*)

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ABSTRACT

Few studies have been done on milk composition of chiropteran species, especially within the family Vespertilionidae. The objective of this research was to describe the milk composition of the pallid bat, *Antrozous pallidus*, a vespertilionid species of bat whose milk composition has yet to be studied. *Antrozous pallidus* milk composition was described using milk samples collected between May and June 2019 in Brewster and Presidio County, Texas. Samples from 13 lactating females were collected, pooled, and analyzed to determine percentages of dry matter, protein, carbohydrate, and fat. *Antrozous pallidus* milk composition was determined to be an average of 24.1% dry matter, 10.1% fat, 9.7% protein, and 3.4% carbohydrates. The low water, high fat, protein, and energy content of this milk is not significantly different from other insectivorous and vespertilionid species, suggesting diet and/or phylogeny may be influencing the milk composition in this study.

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INTRODUCTION

Bats constitute over twenty percent of the approximately 6,500 mammalian species (Burgin et al. 2018). While the ecology of bats has been well studied, very little research has been conducted with regard to their lactation and milk composition, the most energetically demanding aspect of maternal reproduction and one of the primary features that characterizes mammals. Previous milk composition research has focused primarily on bats within the families Phyllostomidae (Huibregtse 1966; Jenness and Studier 1976; Oftedal and Iverson 1995; Stern et al. 1997), and Pteropodidae (Quicke et al. 1984; Messer and Parry-Jones 1997; Korine and Arad 1999; Hood et al. 2001) while very few reports have been published for those species within the family Vespertilionidae, the largest family in order Chiroptera. The few studies that have examined vespertilionids have focused primarily on species of the genus *Myotis* (Kunz et al. 1983; Jenness 1974; Jenness and Studier 1976; Kunz et al. 1995). The underrepresentation of chiropteran species fails to reflect the true diversity within this order and makes it difficult to understand variation in milk composition within the order Chiroptera and across mammalian species. Therefore, to accurately determine the factors that most greatly affect milk composition among species, broader taxonomic representation in milk composition studies is essential. Milk composition data also are critical for future studies identifying the limiting nutritional factors required for growth and development of offspring, but data limited to only a few species will fail to show how these factors vary across species.

Previous studies of lactation in bats have analyzed proximate composition of milk

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(Huibregtse 1966; Jenness 1974; Jenness and Studier 1976; Kunz et al. 1983; Quicke et al. 1984; Oftedal and Iverson 1995; Messer and Parry-Jones 1997; Stern et al. 1997; Korine and Arad 1999; Hood et al. 2001), which quantifies the percentage of protein, fat, carbohydrate, dry matter, and energy that compose the milk. The percent dry matter of the milk, which is the combination of protein, fat, carbohydrate, and ash, is also useful in determining the percentage of water in milk. Other studies have focused primarily on mineral and nitrogen composition (Studier and Kunz 1995; Studier et al. 1995; Stern et al. 1997; Hood et al. 2001; Kwiecinski et al. 2003). However, the variables that influence milk composition and the overall extent of their effect is still largely unclear. Several variables have been hypothesized to influence milk composition in mammals including, suckling frequency (Ben Shaul 1962), maternal size (Tardiff et al. 2001), neonate size (Blaxter 1961), litter size (Tardiff et al. 2001), diet (Jenness and Studier 1976), length of lactation (Skibiel et al. 2013), and phylogeny (Skibiel et al. 2013). Interspecific variation in milk composition has been observed between species of bats with different diets, with insectivorous species producing milk with higher dry matter, fat and protein content compared to omnivorous and frugivorous species (Kunz and Stern 1995), and nectarivorous and frugivorous bats producing milk higher in carbohydrates (Jenness and Studier 1976). However, a recent study that analyzed the milk of 130 different species of mammals suggested that phylogeny is the primary driver of milk composition (Skibiel et al. 2013). This study, which included ten species of bat, used phylogenetic and nonphylogenetic statistical models to determine the effect of biological variables on fat, protein, dry matter and energy density in milk and concluded that phylogeny is the variable with the most significant effect on milk composition. Diet and length of lactation were found to be the second most influential variables affecting the composition of milk (Skibiel et al. 2013). Of the ten bat species included in the Skibiel et al. (2013) study, only two were from the family Vespertilionidae, both of which were of the genus *Myotis*. Vespertilionidae is a large and diverse family of Chiroptera, and restricting the analysis to species within the same genus ultimately limits the conclusions that can be drawn from this analysis in regards to the family Vespertilionidae.

In addition to the limited number of chiropteran species that have been used for milk composition studies, most studies have pooled milk samples from across the lactation period. There are three recognized stages of lactation in bats: early, mid, and late lactation. Early lactation occurs within the first hours or days post-partum, depending on the species, and is characterized by high amounts of colostrum, the first milk secreted from the mammary glands and one that is high in antibodies (Oftedal 1984). Early lactation is also the period in which young are pre-volant with the highest offspring growth rate (Kunz et al. 1995). Mid-lactation is defined as the period of peak milk production when composition is relatively stable (Oftedal 1984). Developmentally, mid-lactation has also been defined as the period in which the growth rate of young begins to decline, and offspring first become volant, but have not yet begun to forage (Kunz et al. 1995). Late-lactation is characterized by declining production of milk as young are weaned to solid foods (Oftedal 1984). Studies observing changes in milk composition throughout lactation, in both chiropteran and other mammalian species, have primarily observed variation in fat, dry matter and energy, while the amount of carbohydrates and protein remain relatively stable. These changes are most prominent among microchiropteran species (Kunz et al. 1995). Ultimately, the changing milk composition observed throughout lactation illustrates the importance of considering stage of lactation during sampling. This is particularly so when comparing milk composition between species as variation in constituents due to lactational stages could spuriously indicate differences in the requirements of offspring. The failure to identify stage of lactation is suspected to contribute to the inconsistencies that occur in milk composition among studies (Kunz et al. 1995). Unfortunately, the stage of lactation can be difficult to determine when sampling from wild populations as time of birth is typically unknown and access to offspring for age determination can be limited.

Antrozous pallidus is one of many vespertilionids for which milk composition has not been described. This species, commonly known as the pallid bat, is a member of the family Vespertilionidae and one of the most abundant bats in the Trans-Pecos region of Texas. The species' range covers mostly arid regions of western North America, starting at the southern border of British Columbia, Canada and continuing south to Jalisco and Querétaro, Mexico (Sidner 1997). The pallid bat is a larger vespertilionid, averaging 12-20 g, and is identified by its bright yellow/blonde pelage and large ears that make it easily distinguishable from other species (Ammerman et al. 2012). Female *A. pallidus* are often larger than males (Ammerman et al. 2012) and commonly give birth to twins, with the likelihood of twin births increasing after the first year of reproduction (Davis 1969, Sidner 1997). Parturition for the species varies with range, but in Texas parturition generally takes place between early-May and mid-June (Ammerman et al. 2012). Within a colony of *A. pallidus*, parturition dates can be as long as two weeks apart (Davis 1969). Post-natal growth of *A. pallidus* offspring is suggested to be slower compared to other studied vespertilionids, and the period of linear growth for this species is reported to be 1 to 22 days post-partum (Bassett 1984). Davis (1969) has reported first flights for developing *A. pallidus* offspring in the wild to be between

33-36 days post-partum, while weaning of offspring is expected to take place between 40-45 days post-partum (Orr 1954).

Like other vespertilionids, *A. pallidus* typically have an insectivorous diet; however, they have also been reported to consume small vertebrates (Lenhart et al. 2010) and feed on the nectar of flowering desert plant species, like *Agave lechuguilla* (Jaquish, 2019) and *Pachycereus pringlei* (Frick et al. 2009). For these reasons they have been characterized as food generalists that also will display opportunistic shifts in their diet. The opportunist shifts observed in the *A. pallidus* diet make this species unique compared to many other insectivorous bats, whose dietary shifts still typically include only invertebrate prey species. The addition of plant resources into the diet of *A. pallidus* provides this species with an additional source of carbohydrates and water unavailable to most other vespertilionids. The additional availability of carbohydrates and water in the *A. pallidus* diet could ultimately affect the overall composition of milk for this species in areas where it is capable of utilizing these resources. The objective of this study was to determine the milk composition of *A. pallidus* in the wild in order to enhance the current understanding of milk among chiropteran species.

Materials and Methods

Sampling Sites

Milk samples were collected from lactating *A. pallidus* from two sites within Big Bend National Park (BBNP), Brewster County, Texas. These capture sites included Ernst Tinaja (29.2562472°, -103.011567°) and Glenn Spring (29.1746139°, -103.1573500°). Ernst Tinaja is a limestone canyon located at the base of the Sierra del Carmen mountains at an elevation of 680 m. The site is characterized as a desert shrub landscape with the most common plant flora including lechuguilla (*Agave lechuguilla*), creosotebush (*Larrea diverticata*), ocotillo (*Fouquieria splendons*), and numerous species of cacti, including, blind prickly-pear (*Opuntia rufida*), and dog cholla (*Opuntia grahamii*) (Wauer 1971; Higginbotham and Ammerman 2002). This site has a tinaja, a large depression in the limestone that fills with runoff and is known to provide a reliable source of water for wildlife, including bats in the area. As such, mist nets were always placed around the perimeter of the tinaja. Glenn Spring is located at an elevation of 780 m and is also characterized as a desert shrub landscape (Wauer 1971; Higginbotham and Ammerman 2002). Unlike Ernst Tinaja, the water source at Glenn Spring is a shallow spring that feeds into the Rio Grande River. Mist nets at this site were placed over shallow pools of water and between areas of dense vegetation or rocky hillsides.

An additional site within BBNP, Mariscal Mine (29.0951666°, -103.1886667°), was also used as a collection site during this study, but no *A. pallidus* were captured at this location. Mariscal Mine is an abandoned mine located at the northern base of Mariscal Mountain at 770 m. A harp trap was placed outside the entrance of a lower mine opening in order to catch bats as they emerged for feeding. The plant flora at this site would also be described as desert shrub, most populated by lechuguilla, creosotebush, and cactus.

Additional samples were collected in Candelaria, Presidio County, Texas on 23-24 June 2019. Captures at this site took place along Capote Creek (30.1697500°, -104.6712222°) at 885 m, small pools were present in the creek at the time of capture and nets were placed over the pools to capture bats as they came to drink.

Sampling Strategy

Sample collection in Brewster County, Texas took place over 10 nights (82.5 net hours) in May and June of 2019. Specifically, netting occurred on the nights of 25-27 May, 30 May, 7-8 June, 10 June, and 21-23 June (Table 1). Sample collection in Presidio County, Texas took place over 2 nights (11.25 net hours) on 23-24 June 2019. These dates were selected to correspond with the period of mid-lactation for *A. pallidus* as parturition for the species is reported to occur from early-May to mid-June in Texas (Ammerman et al. 2012). In addition, incidental captures in BBNP from 1996 through 2018 also suggest that late-May to late-June overlaps with *A. pallidus* lactation (L. Ammerman, unpublished data). Based on these records, it appeared that late May through mid-June would be the ideal time to encounter females that were likely in the stage of mid-lactation as defined by Oftedal (1984) and Kunz et al. (1995). The locations of these recorded encounters were also considered in determining our capture sites, with Glenn Spring and Ernst Tinaja being the most productive sites for *A. pallidus* activity (L. Ammerman, unpublished data).

Collection Methods

Lactating females were captured using mist nets and/or harp traps at each of the capture sites. Capture efforts began at sunset and continued until 1.5 hours after the first lactating female *A. pallidus* was captured. Upon capture, body mass (g) and forearm length (mm) were measured for each lactating female, after which they were held individually in clean canvas bags for no more than 2 hours to allow for refill of mammary glands. Body mass, forearm length, sex, age, and reproductive condition were recorded prior to releasing captured individuals that were not the lactating species of interest. Netting locations at each site and the number of nets used varied according to water availability. The number of nets used for each site ranged from 2-4 in a night.

After allowing time for glandular refill, the female was injected with oxytocin hormone (Henry Schein Animal Health, Dublin, Ohio) 5 minutes prior to the time of milk collection at a recommended dose of 0.4 μl/g (20 IU/mL) to promote the milk letdown response (Hood et al. 2009). The oxytocin was administered intramuscularly into the upper-right pectoralis so as to avoid potential injury to the heart. Prior to expression of the milk, the nipple and surrounding area was sanitized with isopropyl alcohol and a damp cotton ball was placed over the anal-genital area to prevent contamination from urination or defecation. Milk was expressed manually by palpating the mammary gland with gentle and uniform pressure from the thumb and the index finger, starting from the outer perimeter of the gland and moving inward to move the milk toward the nipple. During sanitation and milk collection, the individual collecting the sample wore nitrile gloves to avoid contaminating the sample with oils. A non-heparinized, 10 μl capillary tube (Dade Diagnostics Inc., Miami, Florida) was used to collect the milk upon immediate expression and aided in determining the approximate milk volume collected. After the capillary tube had collected the sample, an end cap was used to expel the sample into a pre-weighed, sterile 0.5 ml Eppendorf tube with an O-ring sealed screw cap to minimize sample evaporation. Smaller volumes of milk (<20 μl) were pooled at the time of sampling in order to minimize the evaporation of the sample as well. Milk was collected from both nipples of each female until both glands had been expressed as completely as possible to avoid systematic bias, as incomplete milking is suspected to produce milk that is lower in fat (Hood et al. 2009). Once the gland had been expressed to completion the sample was diluted with approximately equal amounts of sterile water to minimize the amount of water sublimated from the milk, which can result in issues with sample homogeneity once thawed (Hood et al. 2009). The sample was then immediately placed in liquid nitrogen to be kept frozen until it could be transferred to a -80°C freezer while awaiting analysis. After sample collection, females were released at the capture site. All animal handling protocols were consistent with the guidelines published by the American Society of Mammalogists (Sikes et al. 2016), and were approved by the IACUC committees of Angelo State University (#19-201) and the National Park Service (#IMR_BIBE_Ammerman_Bats_2019.A2). This project was completed under a valid National Park Service permit (#BIBE-2019-SCI-0023) and Texas Parks and Wildlife Scientific Research Permit (#SPR-0994-703).

Sample Analysis

Samples were sent to the Smithsonian's National Zoological Park Nutrition Lab in Washington, D.C. on 12 September 2019 for proximate analysis of the milk. Analysis of the samples began on 10 October 2019 and was completed 2 January 2020. Samples were analyzed for carbohydrates, protein, and percentage of dry matter, and subsequently those results were used to calculate fat percentage. Assay replicates were not run for any of the samples during carbohydrate analysis due to a limited volume of milk, but one set of replicates was used in determining the percentage of dry matter and when conducting protein analysis. Samples were thawed to room temperature and vortexed prior to conducting each method of analysis. Samples that did not meet the minimum volume required to conduct dry matter, protein, and carbohydrate analyses were pooled prior to conducting analysis according to volume and date of collection. The results of dry matter, protein, and carbohydrate analysis were multiplied by two to account for the milk being diluted with equal amounts sterile water at the time of collection.

Dry-matter was determined by calculating changes in the mass of the sample using a Mettler Toledo XPR2 electric balance (+/- 0.001 mg). Subsamples totaling 12 µl were aliquoted into pre-weighed aluminum containers using a 25 µl Finnpipette PDP pipette, and the total mass of sample and container was recorded in mg. The containers holding the sample were transferred into an oven set at 100°C for 4 hours. The samples were then removed from the oven, allowed to cool for 10 minutes and reweighed. After reweighing the dried product, the aluminum container holding the sample was pinched closed and folded into a ball to await its use in nitrogen analysis. The mass of the dried product was used in calculating the percent of dry matter through the division of the final dried mass by the initial mass of the subsample. These methods were outlined in Hood et al. (2009). One set of sample replicates was used for this procedure, and the coefficient of variation was determined for the sample replicates to determine the within-sample variance. Results of the replicate runs were averaged to determine the percent dry matter for each sample.

Carbohydrate analysis of the samples was conducted using phenol-sulfuric acid methods outlined in Hood et al. (2009). This method is sensitive to microgram quantities of sugar in milk and is capable of reliably measuring most milk saccharides, excluding amino saccharides (Hood et al. 2009). Due to the sensitivity of the phenol-sulfuric acid method to quantities of sugar, subsamples were diluted using 15 µl of sample into 20 ml of distilled water to obtain a sugar concentration of approximately 25 µg/ml solution. The mass of sample used in the dilution and the final mass of the diluted product were recorded in grams to determine the final diluted milk concentration. Due to the reliability of this method (Hood et al. 2009) and the limited sample volume, no replicates were run for this analysis. Sample controls were also not used during this process, but a fresh standard curve was run with the samples to ensure accuracy.

Protein composition was determined for the samples using elemental analysis, a combustion method in which organic nitrogen from the milk is converted into a gaseous form and measured by a Carbon-Hydrogen-Nitrogen (CHN) elemental gas analyzer, as summarized in Hood et al. (2009). This method of analysis has proven to be accurate and precise, even for samples with a dry mass of only 1-3 mg (Hood et al. 2009). The dried samples obtained during the assay of percent dry matter were used in this procedure and all dry sample weights were within the 1-3 mg range. Sample replicates produced during the dry matter procedure were used for this procedure as well, and the coefficient of variation was determined for this set of replicates to determine the within-sample variance. Results of the replicate runs were averaged to determine the percent protein in each sample.

Fat composition is typically determined using a Roese-Gottlieb assay that has been micro adapted to analyze smaller samples as described by Hood et al. (2009), but due to limited sample volume, fat analysis was not possible for the set of samples in this study. Fat composition for this study was instead calculated by subtracting the sample percentages of sugar and protein, as well as an assumed 1% of ash, from the percent dry matter that had been determined for each sample (He et al. 2019). The use of an assumed 1% ash content was determined based upon the percent ash reported in previous chiropteran milk composition studies.

Energy content of the milk was calculated for each sample using the determined milk composition and the energy equivalents of carbohydrates (16.5 kJ/g), fat (38.1 kJ/g), and protein (24.5 kJ/g), as outlined in Hood et al. (2009). While this method of determining energy content is common, it is important to acknowledge that the energy equivalents for carbohydrates, fat, and protein used in this calculation were produced by the milks of domestic animals and may not be entirely accurate for chiropteran species (Hood et al. 2009). The percentage of energy contributed by each macronutrient to the gross energy of *A. pallidus* milk was calculated and compared to that of other chiropteran species.

Data Analysis

The average ± standard deviation (*SD*) for dry matter, each macronutrient, and energy content was determined. The average percent carbohydrate, fat, and protein constituting *A. pallidus* milk was compared to the average milk composition for additional chiropteran species that have been used in similar studies. The proportions of carbohydrate, fat, and protein that make up the dry matter of *A. pallidus* milk were also compared to the dry matter proportions of previously reported chiropteran milks. For previous studies that did not determine the percent dry matter, but reported the milk percentage for each macronutrient, the dry matter was calculated from the sum of reported macronutrient percentages and an assumed 1% of ash. For previous studies that did not report the fat percentage of milk, but reported the remaining milk constituents, the amount of fat was calculated from the reported dry matter, carbohydrates, protein, and ash.

A student's two-sample t-test was conducted in R (R Development Core Team, 2012) for each milk variable (dry matter, fat, protein, carbohydrate, and energy) in order to identify significant changes in the variables between the two sampling periods. Prior to conducting the student's two-sample t-test, a Bartlett's test was conducted to determine if the data met the assumption of homoscedasticity and a quantile-quantile plot was examined for each milk variable to ensure the data was normally distributed (McDonald 2014). For variables that did not meet the assumptions of normality, a Welch's t-test was conducted (McDonald 2014). All tests were conducted in R (R Development Core Team, 2012). A *P*-value of less than 0.05 was accepted as significant.

Multivariate statistics (principal components analysis, PCA) were conducted using JMP software (SAS Institute Inc. 2019) to examine how similar the milk composition of chiropteran species was across two classifications: diet and phylogeny. Milk composition data for 18 previously studied bat species were collected from the literature and species data were coded according to their family, based upon the phylogenetic supertree produced by Jones et al. (2002), and diet (frugivore, insectivore, nectarivore, and omnivore) (Cakenberghe et al. 2002; Cole and Wilson 2006). Principal components were constructed as linear combinations of milk constituents (dry matter, fat, protein, carbohydrates, water, and energy), and a biplot was created using the two principal components that accounted for the greatest amount of variance in the data. Each data point was plotted against the two principal components, after which a 95% confidence ellipse was generated to contain all data points in a family or within a particular diet. The size of the ellipses was used to illustrate how each classification (phylogeny or diet) explained the variation in milk composition between species. Wide ellipses indicated great variability in milk composition within a group, and narrow ellipses indicated less variation. The data from this study was then plotted against the principal components to determine if *A. pallidus* data grouped better based on phylogeny or diet.

Results

Capture Data

Over the course of the study, 218 bats were captured within Big Bend National Park (BBNP), Brewster County. *Antrozous pallidus* (28%) and *Myotis thysanodes* (32%) comprised the majority of captures. Multiple *Corynorhinus townsendii*, *Myotis californicus, Myotis velifer, Mormoops megalophylla*, and *Parastrellus hesperus* were captured as well, in addition to a single *Aeorestes cinereus*, *Eptesicus fuscus*, *Leptonycteris nivalis*, and *Tadarida brasiliensis* (Table 1). A total of 61 *A. pallidus* were captured in BBNP (Table 2). These individuals were captured only at Ernst Tinaja and Glenn Spring. Of the 61 *A. pallidus* captured, 35 were captured at Glenn Spring and 26 were captured at Ernst Tinaja. The 35 individuals captured at Glenn Spring were comprised primarily of lactating females (n=19). Similar proportions of lactating females (n=12) and juveniles (n=11) were captured at Ernst Tinaja; however, this was largely due to the increase in juveniles present during the later collection period.

In Candelaria, Texas, a total of 78 bats were captured between 23-24 June 2019. The most encountered bats were *A. pallidus* and *P. hesperus*, but *E. fuscus, M. megalophylla, Myotis velifer, Myotis yumanensis*, and *T. brasiliensis* were captured at this site as well. A total of 21 *A. pallidus* were caught over the two nights, with 16 captured on 23 June and 5 on 24 June. Of the 21 pallid bats, 12 were lactating females, one female was post-lactating, and the remaining individuals were either non-reproductive or undetermined.

Milk Data

The milk of 13 female pallid bats in unknown stages of lactation was collected and analyzed for this study (Table 3). Due to the small volume of milk samples, 11 of the 13

Table 1.—Bat species collected at locations in Brewster Co. and Presidio Co., Texas in 2019. Total number of individuals collected at each sample site in Big Bend National Park and Candelaria, Texas throughout the duration of this study. Glenn = Glenn Spring, Ernst = Ernst Tinaja, Mariscal = Mariscal Mine, and Cand = Candelaria, Texas

Species	25 May (Glenn)	26 May (Mariscal)	27 May (Ernst)	30 May (Glenn)	7 Jun (Glenn)	8 Jun (Mariscal)	10 Jun (Ernst)	21 Jun (Glenn)	22 Jun (Ernst)	23 Jun (Glenn)	23 Jun (Cand)	24 Jun (Cand)
A. cinereus	-	-	-	-	-	-	-	-	1	-	-	-
A. pallidus	18	-	9	4	-	-	-	4	17	9	16	5
C. townsendii	4	7	-	-	-	12	-	-	-	3	-	-
E. fuscus	-	-	-	-	-	-	-	-	-	1	3	2
L. nivalis	-	-	-	-	-	-	-	-	-	1	-	-
M. megalophylla	1	-	-	-	2	-	-	1	-	-	1	1
M. californicus	1	-	-	-	1	-	-	2	4	-	-	-
M. thysanodes	-	51	-	-	-	20	-	-	-	-	-	-
M. velifer	3	10	-	-	1	5	-	-	2	3	1	-
M. yumanensis	-	-	-	-	-	-	-	-	-	-	-	2
P. hesperus	3	-	6	-	-	-	-	1	9	1	22	20
T. brasiliensis	1	-	-	-	-	-	-	-	-	-	2	3

Table 2.—Total number of *A. pallidus* individuals collected at each sample site in Big Bend National Park and Candelaria, Texas in 2019, as well as the reproductive condition for each individual. Glenn = Glenn Spring, Ernst = Ernst Tinaja, Mariscal = Mariscal Mine, and Cand = Candelaria, Texas

Condition	25 May (Glenn)	27 May (Ernst)	30 May (Glenn)	7 Jun (Glenn)	10 Jun (Ernst)	21 Jun (Glenn)	22 Jun (Ernst)	23 Jun (Glenn)	23 Jun (Cand)	24 Jun (Cand)	Total
Lactating	14	9	3	-	-	1	3	1	9	3	43
Post-lactating	-	-	-	-	-	-	-	-	1	-	1
Pregnant	2	-	-	-	-	-	-	-	-	-	2
Scrotal	-	-	-	-	-	-	2	1	-	-	3
Non-reproductive	-	-	-	-	-	-	-	-	-	-	-
Adult, Female	-	-	1	-	-	-	-	-	2	-	3
Adult, Male	1	-	-	-	-	-	1	-	2	-	4
Juvenile	-	-	-	-	-	3	11	7	-	-	21
Undetermined	1	-	-	-	-	-	-	-	1	-	2
Female	-	-	-	-	-	-	-	-	-	-	0
Male	-	-	-	-	-	-	-	-	1	2	3

samples were pooled to meet the minimum volume required to conduct analyses. Samples were pooled according to the sample volume and period of collection (Table 3) in order to account for the possibility of varying stages of lactation over the study's duration. Samples with a volume of less than 65 µl were pooled into one of two time periods: 27-30 May or 21-24 June. Samples that met the minimum 65 µl requirement for analysis were analyzed individually. After pooling samples based on volume and collection period (early and late), a total of 4 samples remained, with each collection period producing 1-pooled sample and 1 individual sample (Table 3). Proximate analysis of the four samples revealed that the milk of *A. pallidus* was made up of an average of 24.1% dry matter (Table 4), which was comprised of 3.4% carbohydrates, and nearly equal proportions of fat (10.1%) and protein (9.7%). The gross energy content of the milk was determined to be 6.8 kJ/g (Table 4).

In order to identify within-sample variation, the coefficient of variation was determined for the dry matter and protein of each sample as sample replicates were used during the analysis for both constituents. The coefficient of variation for the dry matter of samples S1-S4 was determined to be 0%, 7.2%, 0.4%, and 13.7%, respectively. The coefficient of variation for the protein content of samples S1-S4 was determined to be 0.6%, 0.3%, 0.07%, and 1.9%, respectively. According to Hood et al. (2009), the within-sample variance is considered normal if the coefficient of variance does not surpass 5%, in which case additional replicates would be ideal. Based on these standards, the amount of within-sample variation for the protein content of each sample and the dry matter content for samples S1 and S3 are considered normal; however, the amount of variation in dry matter for replicates of samples S2 and S4 show that the dry matter composition for these samples may not be entirely reliable.

Table 3.—Measurements, collection location, and estimated volume of milk collected from each individual female *Antrozous pallidus* used in this study.

Collection Date	Weight (g)	Forearm length (mm)	Volume Milk Collected (μl)	Collection Site	Sample ID
27 May	14.0	49.1	< 10.0	Ernst Tinaja	S2
27 May	16.0	50.8	< 10	Ernst Tinaja	S2
30 May	14.0	47.7	12	Glenn Spring	S2
30 May	12.5	48.8	15	Glenn Spring	S2
30 May	16.5	49.2	65	Glenn Spring	S1
21 June	17.5	51.1	76	Glenn Spring	S3
22 June	16.5	51.0	10	Ernst Tinaja	S4
22 June	14.5	50.8	6	Ernst Tinaja	S4
22 June	15.0	53.5	35	Ernst Tinaja	S4
23 June	14.0	49.7	9	Glenn Spring	S4
23 June	16.0	50.3	5	Candelaria	S4
23 June	18.0	52.4	12	Candelaria	S4
23 June	17.0	55.8	5	Candelaria	S4

Table 4.—Proximate analysis results for pooled and unpooled samples of milk from *Antrozous pallidus* captured in the wild in 2019. Average milk composition and standard deviation (SD) was calculated using all samples. *N* indicates the number of milk samples collected from separate bats that were pooled to form the sample. Carb = carbohydrate.

Sample	Dry Matter (% wet mass)	Fat (% wet mass)	Protein (% wet mass)	Carb. (% wet mass)	Energy (kJ/g)
S1—30 May (*N*=1)	19.2	7.0	7.5	3.7	5.1
S2—27-30 May (*N*=4)	22.8	8.0	10.4	3.4	6.2
S3—21 June (*N*=1)	27.7	13.1	10.3	3.3	8.1
S4—21-24 June (*N*=7)	26.6	12.1	10.5	3.0	7.7
Average	24.1	10.1	9.7	3.4	6.8
SD	±3.89	±3.00	±1.45	±0.29	±1.38

While the stage of lactation could not be determined for individuals used in this study, differences were observed between samples collected during the early collection period in May compared to those collected in June (Fig. 1). Samples taken during the later collection period were observed to have a higher percentage of fat and dry matter, and therefore less water, compared to those collected earlier in the season. Samples collected in June were observed to be 6.2% higher in dry matter and 5.1% higher in fat compared to samples collected in May (Fig. 2). Samples collected in June were also observed to be 1.4% higher in protein and 0.4% lower in carbohydrates, while energy increased by 2.3 kJ/grams. Based on a student's two-sample t-test, it was determined that observed differences in average fat composition between early and late sample sets were significantly different ($t=-7.21$, $df=2$, $P=0.02$), while the amounts of dry matter ($t=-3.27$, $df=2$, $P=0.08$), carbohydrates ($t=1.89$, $df=2$, $P=0.20$), and energy ($t=-3.84$, $df=2$, $P=0.06$) were not significant. Protein data were not normally distributed, therefore, a Welch's t-test was used, the results of which illustrated that protein ($t=-1.00$, $df=1$, $P=0.50$) did not significantly increase.

Comparative Analysis of Bat Milk

Milk composition of *A. pallidus* differed from other bats (Fig. 3). The percentage of constituents that make up the milk in its entirety occur at intermediate amounts compared to other species, for which the percentage of constituents making up the milk ultimately varied across all species for dry matter (11.1-40.5%), fat (1.9-29.0%), protein (1.9-12.1%), and carbohydrates (2.5-7.3%) (Table 5). When comparing the composition of whole milk, it is

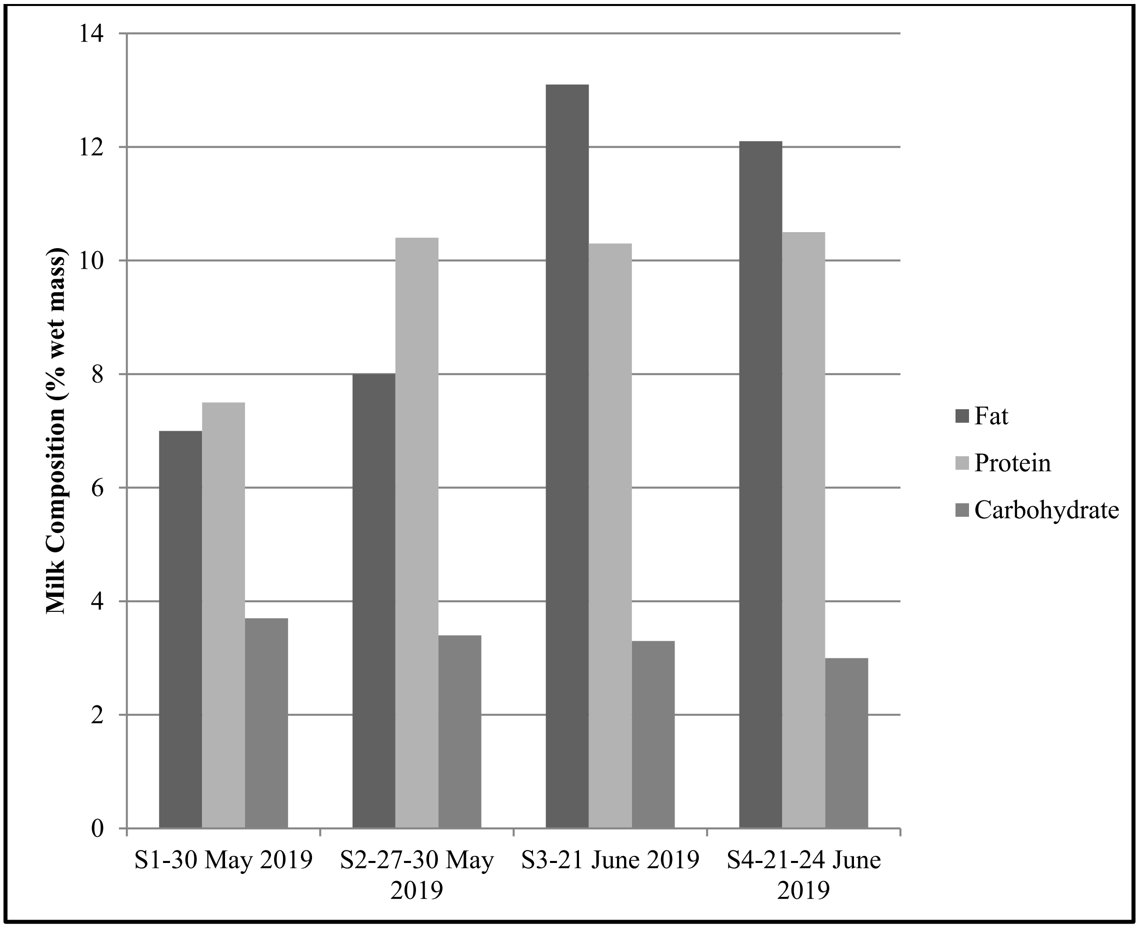

Fig. 1.—Seasonal comparison of *A. pallidus* milk composition within Big Bend National Park and Candelaria, Texas in May and June 2019.

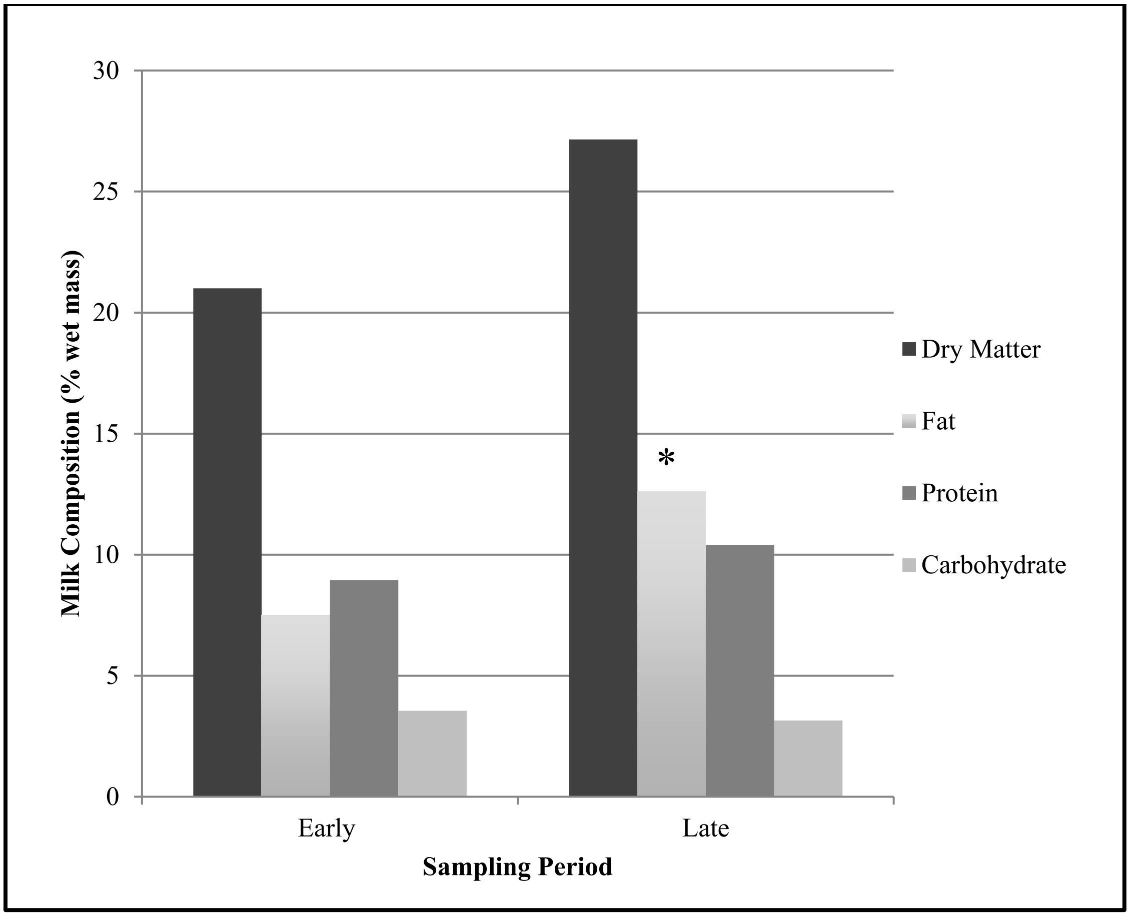

Fig 2.—Comparison of average *A. pallidus* milk composition between early and late sampling periods taken in Big Bend National Park and Candelaria, Texas in May and June 2019. Fat levels in June were significantly different (*) from levels in May.

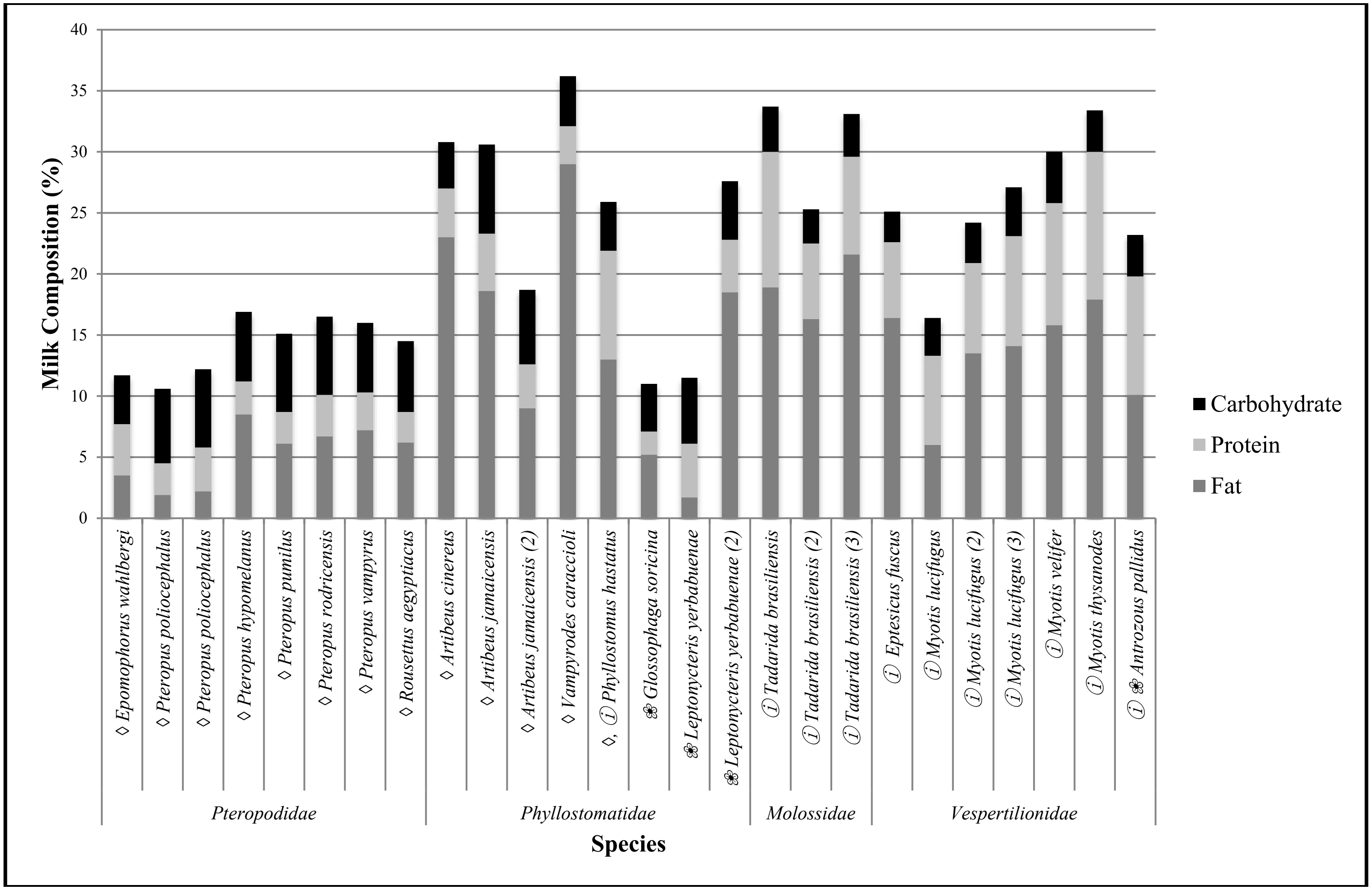

Fig. 3.—Average proportions of macronutrients comprising dry matter of bat milk. Averages of milk composition were used for studies that listed multiple values for the same macronutrient, specifically those that reported constituents throughout lactation stages. Repeated species indicate a species' milk has been analyzed in more than one study. Icons preceding the species indicate the species' diet, "◇" indicates the species is frugivorous, "❀ " indicates nectarivorous, and "ⓘ" indicates insectivorous. Any combination of icons indicates the species utilize both food sources

difficult to establish patterns in the milk composition between species, but similarities can be observed in the proportions of carbohydrate, protein, and fat that make up the dry matter of *A. pallidus* milk when compared to the dry matter content of other vespertilionids and *Phyllostomus hastatus*, an omnivorous species of bat (Fig. 3). Additional similarities can be observed when comparing the average percent energy contributed by carbohydrates to the gross energy of the milk, for which *A. pallidus* (8.3%) and other insectivorous (4.9-11.1%) milks were not only lower than that of nectarivorous and frugivorous milks (5.4-45.2%), but the range was also much narrower (Table 6). When comparing the average amount of energy contributed by each macronutrient to the total gross energy for insectivorous/omnivorous versus frugivorous/nectarivorous species, the average percent energy contributed by carbohydrates was lower in insectivorous/omnivorous species (7.2%) compared to frugivorous/nectarivorous species (22.6%), while the percent protein was higher for insectivorous/omnivorous species (26.5%) compared to frugivorous/nectarivorous (19.8%). While ultimately higher in insectivorous species, the energy from fat varied greatly between diet types, resulting in a smaller difference in the averages of energy from fat in insectivorous (67.1%) and non-insectivorous species (62.0%).

Principal Component Analysis

In order to determine if similarities in milk composition were based on phylogeny and/or diet, data from previous lactation studies (Table 5) were used to conduct Principal Component Analysis (PCA). The first two principal components produced by PCA accounted for 91.9% of the milk composition variation observed among studies (Fig. 4). Component 1 was positively correlated with fat energy, and dry matter, with eigenvector values of 0.43411, 0.45542, and 0.45553, respectively, and negatively correlated with water,

Table 5.—The average percentage each milk constituent contributes to the overall wet mass of the milk as reported by previous studies of chiropteran milks, in addition to values reported in this study for *Antrozous pallidus*. Subscripts "*W*" and "*C*" follow species name to indicate if the population was wild-caught (*W*) or captive I.
[a]References: 1. Huibregtse 1966; 2. Jenness 1974; 3. Jenness and Studier 1976; 4. Kunz et al. 1983; 5. Quicke et al. 1984; 6. Kunz et al. 1995; 7. Oftedal and Iverson 1995; 8. Messer and Parry-Jones 1997; 9. Stern et al. 1997; 10. Korine and Arad 1999; 11. Hood et al. 2001

Family	Species	Dry Matter	Fat	Protein	Carbohydrate	Ref.[a]
Pteropodidae	*Epomophorus wahlbergi* $_{W,C}$	12.0	3.5	4.2	4.0	5
Pteropodidae	*Pteropus poliocephalus* $_{C}$	11.1	1.9	2.6	6.1	8
Pteropodidae	*Pteropus poliocephalus* $_{W}$	12.7	2.2	3.6	6.4	8
Pteropodidae	*Pteropus hypomelanus* $_{C}$	18.5	8.5	2.7	5.7	11
Pteropodidae	*Pteropus pumilus* $_{C}$	16.2	6.1	2.6	6.4	11
Pteropodidae	*Pteropus rodricensis* $_{C}$	19.1	6.7	3.4	6.4	11
Pteropodidae	*Pteropus vampyrus* $_{C}$	16.5	7.2	3.1	5.7	11
Pteropodidae	*Rousettus aegyptiacus* $_{W}$	15.5	6.2	2.5	5.8	10
Phyllostomatidae	*Artibeus cinereus* $_{W}$	31.8	23.0	4.0	3.8	3
Phyllostomatidae	*Artibeus jamaicensis* $_{W}$	31.6	18.6	4.7	7.3	3
Phyllostomatidae	*Artibeus jamaicensis* $_{C}$ (2)	17.8	9.0	3.6	6.1	7
Phyllostomatidae	*Vampyrodes 18araccioli* $_{W}$	37.2	29.0	3.1	4.1	3
Phyllostomatidae	*Phyllostomus hastatus* $_{W}$	25.7	13.0	8.9	4.0	9
Phyllostomatidae	*Glossophaga yerbabuenae* $_{W}$	12.0	5.2	1.9	3.9	3
Phyllostomatidae	*Leptonycteris yerbabuenae* $_{W}$	12.1	1.7	4.4	5.4	1
Phyllostomatidae	*Leptonycteris sanborni* $_{W}$ (2)	28.6	18.5	4.3	4.8	3
Molossidae	*Tadarida brasiliensis* $_{W}$	34.4	18.9	11.1	3.7	1
Molossidae	*Tadarida brasiliensis* $_{W}$ (2)	26.3	16.3	6.2	2.8	3
Molossidae	*Tadarida brasiliensis* $_{W}$ (3)	32.8	21.6	8.0	3.5	6
Vespertilionidae	*Eptesicus fuscus* $_{W}$	26.1	16.4	6.2	2.5	4
Vespertilionidae	*Myotis lucifugus* $_{W}$	17.4	6.0	7.3	3.1	3
Vespertilionidae	*Myotis lucifugus* $_{W}$ (2)	25.2	13.5	7.4	3.3	4
Vespertilionidae	*Myotis lucifugus* $_{W}$ (3)	26.8	14.1	9.0	4.0	6
Vespertilionidae	*Myotis velifer* $_{W}$	28.9	15.8	10.0	4.2	6
Vespertilionidae	*Myotis thysanodes* $_{W}$	40.5	17.9	12.1	3.4	2
Vespertilionidae	*Antrozous pallidus* $_{W}$	24.1	10.1	9.7	3.4	this study

Table 6.—The average percent energy contributed by each milk constituent to the gross energy of the milk as reported by previous studies of chiropteran milks, in addition to values reported in this study for *Antrozous pallidus*. Subscripts "*W*" and "*C*" follow species name to indicate if the population was wild-caught (*W*) or captive I. Carb = Carbohydrate, GE=Gross Energy.
[a]References: 1. Huibregtse 1966; 2. Jenness 1974; 3. Jenness and Studier 1976; 4. Kunz et al. 1983; 5. Quicke et al. 1984; 6. Kunz et al. 1995; 7. Oftedal and Iverson 1995; 8. Messer and Parry-Jones 1997; 9. Stern et al. 1997; 10. Korine and Arad 1999; 11. Hood et al. 2001

Diet	Species	Fat	Protein	Carb	Total GE (kJ/g)	Ref.[a]
Frugivore	*Epomophorus wahlbergi* $_{W,C}$	44.5	34.3	22.0	3.0	5
Frugivore	*Pteropus poliocephalus* $_{C}$	30.2	26.5	41.9	2.4	8
Frugivore	*Pteropus poliocephalus* $_{W}$	31.0	32.7	39.1	2.7	8
Frugivore	*Pteropus hypomelanus* $_{C}$	81.0	16.5	23.5	4.0	11
Frugivore	*Pteropus pumilus* $_{C}$	68.4	18.7	31.1	3.4	11
Frugivore	*Pteropus rodricensis* $_{C}$	56.7	18.5	23.5	4.5	11
Frugivore	*Pteropus vampyrus* $_{C}$	78.4	21.7	26.9	3.5	11
Frugivore	*Rousettus aegyptiacus* $_{W}$	59.1	15.3	23.9	4.0	10
Frugivore	*Artibeus cinereus* $_{W}$	84.3	9.4	6.0	10.4	3
Frugivore	*Artibeus jamaicensis* $_{W}$	75.4	12.3	12.8	9.4	3
Frugivore	*Artibeus jamaicensis* $_{C}$ (2)	64.7	16.6	19.0	5.3	7
Frugivore	*Vampyrodes 18araccioli* $_{W}$	88.4	6.1	5.4	12.5	3
Omnivore	*Phyllostomus hastatus* $_{W}$	64.3	28.3	8.6	7.7	9
Nectarivore	*Glossophaga soricina* $_{W}$	63.9	15.0	20.8	3.1	3
Nectarivore	*Leptonycteris yerbabuenae* $_{W}$	24.9	41.5	34.3	2.6	1
Nectarivore	*Leptonycteris yerbabuenae* $_{W}$ (2)	79.2	11.8	8.9	8.9	3
Insectivore	*Tadarida brasiliensis* $_{W}$	68.6	25.9	5.8	10.5	1
Insectivore	*Tadarida brasiliensis* $_{W}$ (2)	75.7	18.5	5.6	8.2	3
Insectivore	*Tadarida brasiliensis* $_{W}$ (3)	78.4	18.7	5.5	10.5	6
Insectivore	*Eptesicus fuscus* $_{W}$	74.4	18.1	4.9	8.4	4
Insectivore	*Myotis lucifugus* $_{W}$	49.7	38.9	11.1	4.6	3
Insectivore	*Myotis lucifugus* $_{W}$ (2)	70.5	24.8	7.5	7.3	4
Insectivore	*Myotis lucifugus* $_{W}$ (3)	67.2	27.6	8.3	8.0	6
Insectivore	*Myotis velifer* $_{W}$	67.6	27.5	7.8	8.9	6
Insectivore	*Myotis thysanodes* $_{W}$	65.6	28.5	5.4	10.4	2
Insectivore	*Antrozous pallidus* $_{W}$	56.6	34.9	8.3	6.8	this study

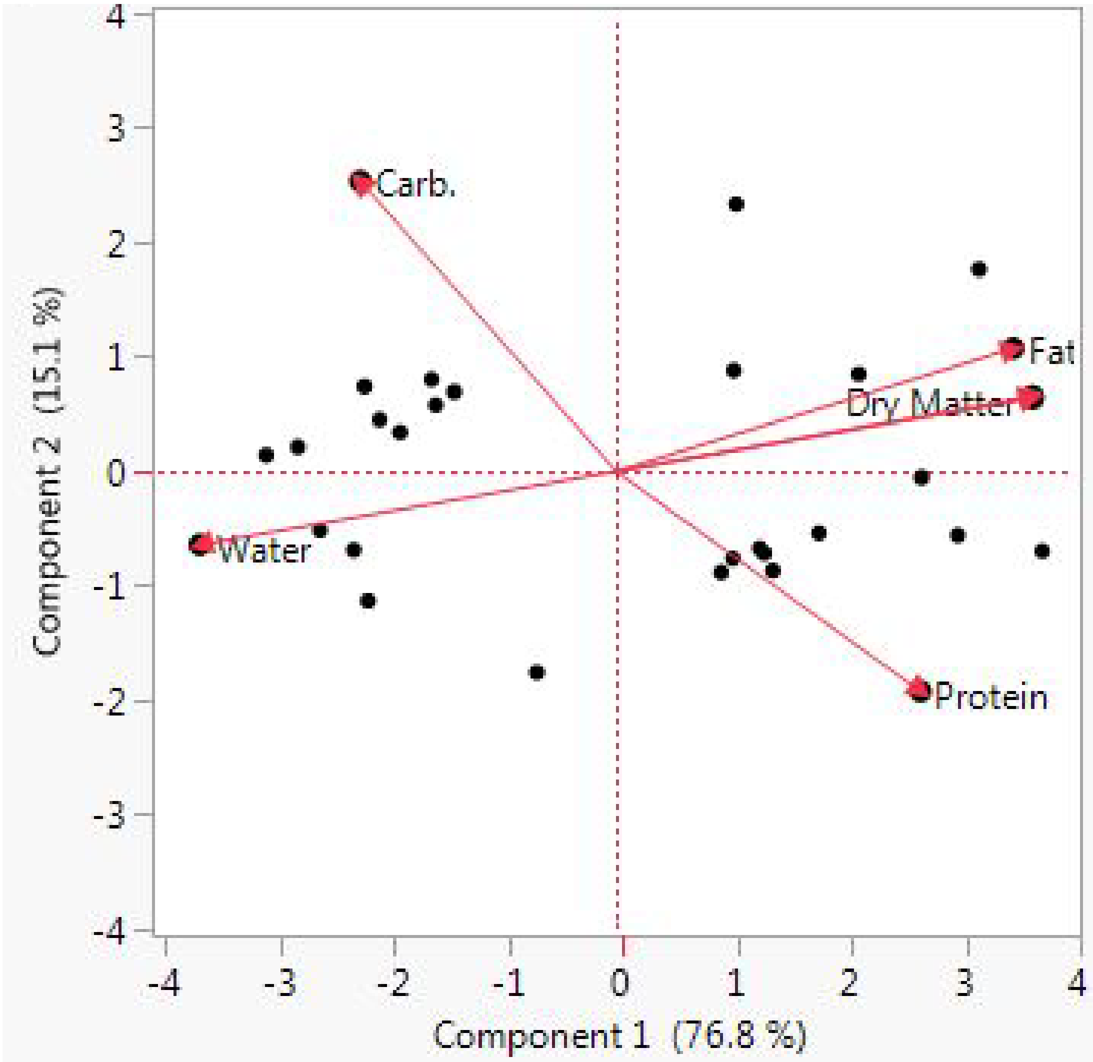

Fig. 4.—Results from the principal component analysis for the milk composition data of previously studied chiropteran species displayed as a biplot.

with an eigenvector value of -0.45553. Component 1 accounted for 76.8% of the variability within the milk composition dataset of previously reported species and had an eigenvalue of 4.6105. Component 2 was negatively correlated with protein and positively correlated with carbohydrate, with eigenvector values of -0.54243 and 0.71665, respectively. Component 2 accounted for 15.1% of the variability in the dataset and had an eigenvalue of 0.9058.

When displaying the 95% confidence ellipses as a function of family type (Fig. 5), the larger width of the ellipse for family Phyllostomidae showed this family had the greatest amount of variability in milk composition. Overlapping ellipses for families Phyllostomidae and Vespertilionidae was due to the placement of the *Phyllostomus hastatus* datapoint. The placement of *A. pallidus* and other vespertilionids on the biplot produced by PCA shows that this milk is characterized as being higher in fat, protein, energy, and dry matter and lower in carbohydrates and water (Fig. 4). When displaying the 95% confidence ellipses as a function of diet type (Fig. 6), the milk composition of *A. pallidus* fell within the confidence ellipses that described an insectivorous diet, which when displayed on the biplot, also characterized the milk as being higher in fat, protein, energy, and dry matter and lower in carbohydrates and water (Fig. 4). The insectivorous ellipse was also shared by the datapoint for *P. hastatus*, an omnivorous species.

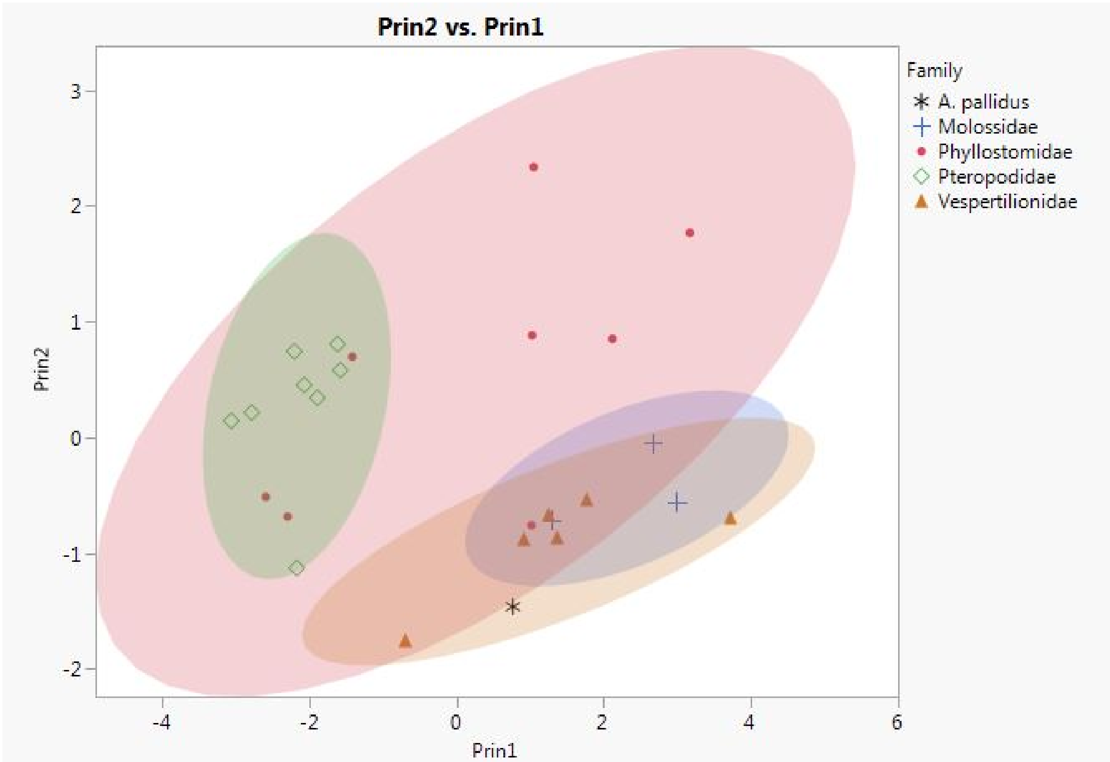

Fig. 5.—Results from the principal component analysis for the milk composition data of previously studied chiropteran species (listed in Table 5), overlaid with 95% confidence ellipses to segregate data point clusters based on family.

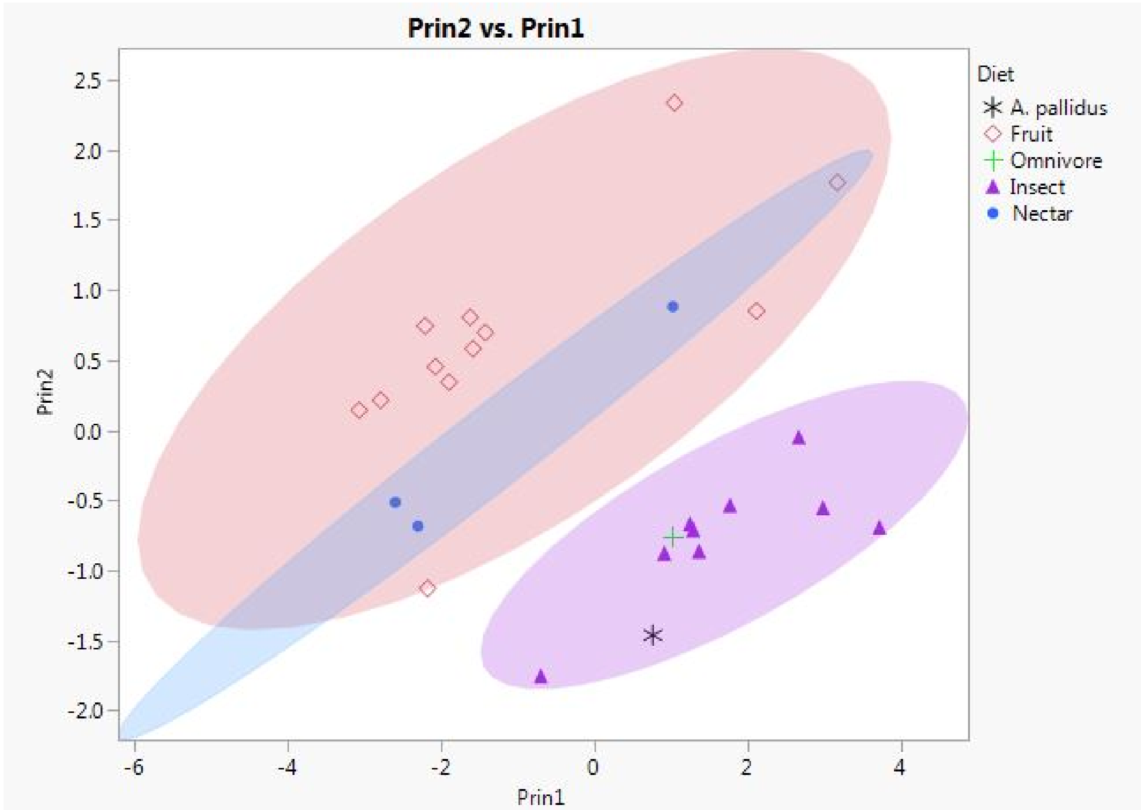

Fig. 6.—Results from the principal component analysis for the milk composition data of previously studied chiropteran species (listed in Table 5), overlaid with 95% confidence ellipses to segregate data point clusters based on diet.

DISCUSSION

Milk Description

My results suggest that the milk composition of *A. pallidus* is of moderate nutrient density, which is typical for most chiropteran species. The milk of *A. pallidus* is also observed to be low in carbohydrates, with high, and equitable amounts of fat and protein, as defined by Oftedal (1984). The high fat composition allows the milk to not only be higher in dry matter, but also results in a milk that is higher in energy content and less dilute.

Changes in the milk composition of *A. pallidus* were observed over time, with fat significantly increasing in samples collected later in the study. The observed increase in fat across time is not entirely unexpected, as increases in fat later in lactation have been observed in other chiropteran (Kunz et al 1995, Hood et al. 2001) and non-chiropteran species (Arnould and Hindell 1999; Laudenslager et al. 2010; Power et al. 2018). This increase in fat composition could be explained by increasing metabolic demands of the offspring, as well as the need for extra calories to meet the energetically expensive requirements of flight as they learn to forage on their own. The latter of these explanations is supported by observations made by Manning et al. (1987), in which the intestinal contents of juvenile pallid bats were reported to contain a mixture of milk and insects. This change in milk composition over time could also be due to maternal water conservation. By late-lactation young have grown and have a lower surface area to body volume ratio, which reduces their rate of evaporative water loss. Additionally, young may be capable of obtaining water through other sources, including free water and moist foods.

It should be noted, without knowing parturition date for each lactating female, the stage of lactation for individuals used in this study cannot be accurately identified. The pooled samples of *A. pallidus* milk between individuals of unknown stages of lactation means caution should be taken in interpreting these results, as milk composition has been shown to be affected by the stage of lactation (Hood et al. 2009). However, the differences in encounter frequency of lactating *A. pallidus* between May and June suggest lactation synchrony among individuals in this study. In addition, the samples that were unpooled for analysis, of which there was one for each collection period, show similar changes in milk composition over time to those of the pooled samples, which further supports the possibility of synchronous reproductive conditions. However, the extent to which synchronous stages of lactation occur between different sites within BBNP is uncertain, and the extent to which synchrony occurs within the Trans-Pecos region is even less certain. Therefore, there is no way of knowing for certain that samples used in this study accurately reflect the composition of *A. pallidus* milk during specific stages of lactation.

Interspecific Variation in Milk Composition

My results show that the average milk composition of *A. pallidus* varied compared to previously studied chiropteran species but, based on PCA results, it does appear to be most similar to other vespertilionids and insectivorous species that have been studied to date. These milks were characterized as being low in carbohydrates and water, and high in fat, protein, dry matter, and energy. However, because vespertilionids and molossids are almost entirely insectivorous, the effect of diet and phylogeny are confounded. While this result was expected, e.g., (Skibiel et al. 2013), the exact placement of *A. pallidus* along the biplot of principal components was further away from other nectarivorous species than expected. Whereas *A. pallidus* is largely insectivorous, with only opportunistic feeding on agave nectar in this region, if diet had a strong effect on milk composition, then the addition of nectar would result in a milk that is more dilute and higher in sugar compared to other insectivores. This was not observed. This may suggest that diet does not influence milk composition as much as phylogeny, but once again this is difficult to determine because vespertilionids are inherently insectivorous. In addition, it is possible that the individuals used in this study did not utilize agave nectar or that the consumption of insects may have outweighed any effect of nectarivory. The consumption of insects outweighing the effects of a nectarivorous diet is also suggested by the location of the *P. hastatus* datapoint, an omnivorous species, that is positioned within the insectivore ellipse.

The nutritional density of *A. pallidus* milk is similar to most other chiropteran species in that it would be characterized as a moderately nutrient dense milk when compared to other mammals (Oftedal 1984). While interspecific variation in dry matter (DM) and gross energy (GE) is observed between chiropteran species, most bat species fall outside the range of dilute milk (DM ≤ 12%, GE ≤ 3.34 kJ/g) and concentrated milk (DM ≥ 30%, GE ≥ 30%) as defined by Oftedal (1984). For comparison, dilute milk occurs more often in primates and perissodactyls, which have a higher proportion of carbohydrates (5.9-8.9%) in the milk compared to fat (0.2-10.2%) and protein (1.1-5.2%) (Oftedal 1984). The amount of dry matter (8.8-19.4%) in the milk is reduced overall compared to more concentrated and moderately nutrient dense milks due to the higher amount of water. The low levels of milk protein observed in these species has been suggested to be correlated with the slow growth rates of offspring (Power et al. 2002). Concentrated milks are associated with more aquatic species, such as pinnipeds and cetaceans, as well as lagomorphs, egg-laying mammals, and bears. This milk composition is very high in dry matter (31.2-69.8%), fat (14.1-61.1%), and protein (4.9-15.8%) with very low amounts of water and carbohydrates (0.02-3.7%) (Oftedal

1984). The energetically dense and concentrated nature of these milks is suggested to be due to the high energetic requirements of the young, to help young accumulate subcutaneous fat stores that provide protection in a thermally demanding environment (Oftedal 1984) or for periods of fasting (Oftedal et al. 1993). The moderate nutrient density of the milk in chiropteran species is more similar to that of many terrestrial carnivorous species, artiodactyls, marsupials, and elephants, which are characterized by moderate amounts of dry matter (9.5-30.6%), fat (3.0-14.3%), protein (4.0-11.0%), and carbohydrates (3.0-12.5%) (Oftedal 1984). The moderate nutrient density of chiropteran milks is thought to be beneficial for fueling the energetic requirements of chiropteran offspring, while the reduction in water compared to more dilute milks is thought to reduce the wing loading of lactating females (Kunz et al. 1995).

While the findings from this study are useful as a baseline indicator of milk composition in *A. pallidus*, they should be considered preliminary. To determine the milk composition of *A. pallidus* with more certainty, future studies of this species should account for stage of lactation and aim to collect samples from more individuals and samples of higher volume to avoid pooling of samples. Larger sample volumes would allow for performance of micro-adapted Roese-Gottlieb assay methods that would more accurately describe the fat composition of *A. pallidus* milk. Although the results of this study should be considered preliminary, the data presented in this study are the first attempt to describe the milk of *A. pallidus*. As the first description of *A. pallidus* milk composition, the results of this study may aid future studies of this kind in determining variables that affect milk composition through the broadened understanding of interspecific variation in milk composition. Contributing such data is also essential for understanding the reproductive strategies used by bats and other mammals (Power et al. 2002). Additionally, the information presented here allows for the appropriate selection of milk formula for *A. pallidus* neonates raised in captivity.

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APPENDICES

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

01/21/2019

Loren K. Ammerman, Ph.D.
Professor of Biology
Curator of Tissues, ASNHC
Angelo State University
ASU Station #10890
San Angelo, TX 76909

Dear Dr. Ammerman:

Your proposed project titled, "Macronutrient composition of milk in six species of insectivorous bat from the Chihuahuan desert" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 01/21/2019, and it expires three years from this date. An annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project will be due on the anniversary of the effective date. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 19-201. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Steven T. Brewer, Ph.D.
Assistant Professor,
Co-Chair, Institutional Animal Care and Use Committee
Director, MS Program in Experimental Psychology
Psychology & Sociology
Angelo State University
Member, Texas Tech University System
ASU Station #10907
San Angelo, TX 76909-0907

Appendix I. – Approval letter written by Dr. Steven Brewer, Co-Chair of the Angelo State University Institutional Animal Care and Use Committee (IACUC), on 21 January 2019. This IACUC approval (#19-201) was for the handling procedures of milk collection from lactating *Antrozous pallidus* females.

Institutional Animal Care and Use Committee

U.S. Department of Interior
National Park Service
Natural Resource Stewardship and Science
Biological Resources Division
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525

Animal Research Protocol Approval Concurrence

IMR_BIBE_Ammerman_Bats_2019.A2

Approval Date: February 20, 2019 **Expiration Date:** January 21, 2022	**Principal Investigator(s):** Loren Ammerman **E-mail:** loren.ammerman@angelo.edu **Telephone:** 325-486-6643
1st **Annual Review Date:** February 20, 2020	**Primary, Approving IACUC:** Angelo State University
2nd **Annual Review Date:** February 20, 2021	**Protocol #:** 19-201 **Approval Range:** 1/21/2019-1/21/2022

Project Title: Macronutrient composition of milk in six species of insectivorous bat from the Chihahuan desert

Funding Agency(ies): Head of the River Ranch Grant, Fazlur & Jahanara Rahman Family Research Grant

This project study was reviewed by the National Park Service Institutional Animal Care and Use Committee. The following action(s) were taken:

Project Status: Concurrence with the primary, approving IACUC of Record
Conditions for Approval: ☒ None ☐ Yes, see below

IACUC Chair and Attending Veterinarian:
Date: February 20, 2019

Note: *Immediately report any/all unexpected mortalities to the NPS IACUC as you would your primary, approving IACUC of record.*

Appendix II. – Project approval document issued by the National Park Service Institutional Animal Care and Use Committee (IACUC), on 20 February 2019. This IACUC approval (#IMR_BIBE_Ammerman_Bats_2019.A2) was for the handling procedures of milk collection from lactating *Antrozous pallidus* females.

BIOGRAPHY

Miranda Marie Perry was born 25 April 1991 in Hamilton, Ohio to William and Carol Perry. From a young age, Miranda planned to pursue a post-secondary education that would allow her to work with animals. In 2008, she was accepted into The Ohio State University as an animal sciences major, where she attended her first two years of college from September of 2009 to June of 2011. In June 2011, she transferred to Miami University in pursuit of her bachelor's degree in zoology. At Miami University, Miranda had her first experience working in research, holding a position as a lab assistant to Patrick M. Mineo. After graduating from Miami University with a Bachelor of Arts in May 2014, Miranda worked various jobs within the field of biology, including an Animal Encounters Ambassador at the Cincinnati Zoo and Botanical Gardens, a veterinary assistant, and a laboratory technician at the DNA Diagnostic Center. Four years after graduating from Miami University, Miranda applied to attend Angelo State University in pursuit of a M.S. degree in biology, to which she was accepted in May 2018. Miranda has since been pursuing a Master of Science degree from Angelo State University's Biology department while completing her thesis work regarding the milk composition of pallid bats.